

Analysis of phenotypic and genetic parameters in a panel of recombinant antibody expressing GS-CHO cell lines

Lonza

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ABSTRACT

Mammalian cell culture is the technology of choice for the manufacture of most therapeutic antibodies. Typically, transfection of mammalian cells results in a heterogeneous population with respect to growth and productivity kinetics. From this population, cell lines with desired growth and productivity traits must be selected for the manufacture of the antibody. Strategies for selecting cell lines with desired characteristics tend to be lengthy and resource intensive: two factors which need to be reduced. A better understanding of the correlation between growth and productivity kinetics and other parameters will help to improve such selection strategies.

A panel of 11 GS-CHO cell lines encompassing a range of productivities were assessed, in triplicate, in a scale-down model of Lonza Biologics' production bioreactor process, using chemically-defined, animal component-free (CDACF) media and feeds. Along with growth and productivity, samples taken from the cultures were also used to assess cell size, nutrient utilisation and levels of mRNA. Copy number for the cell lines in the panel was also assessed.

Growth and productivity of the panel was wide ranging: productivity ranged from 162 mg/L to 1861 mg/L and maximum viable cell concentration (max X_v) ranged from 6.53×10^6 viable cells/mL to 13.65×10^6 viable cells/mL. No correlation was seen between product concentration and glucose utilisation or glutamate utilisation rates. No obvious trend existed between copy number and productivity. The results did suggest that there was a correlation between productivity and both heavy chain mRNA and light chain mRNA expression levels. Higher levels of mRNA were associated with higher productivity. This suggests that transcription is probably a rate limiting step for productivity for the majority of the panel of GS-CHO cell lines.

INTRODUCTION

When host cell lines are transfected with recombinant DNA, variability in parameters associated with the production of this recombinant protein are seen between the resultant recombinant cell lines. Variability is also observed between parameters which are not associated with the production of the recombinant protein. For example, the way in which cell lines respond to their environment (Brand *et al*, 1994). A strategy for selecting a cell line with desired growth and productivity characteristics is therefore required. Such strategies tend to be lengthy and resource intensive. The aim of this study was to identify any correlation between productivity kinetics and various other parameters. A better understanding of any correlation may help to improve selection strategies.

METHODS

Cell Lines

Cell lines were generated by transfecting the host cell line CHOK1SV with the GS vector pEE12.4 containing the gene for the model antibody cB72.3 (E and Z cell lines), or an empty pEE14.4 GS vector (Null 8). The panel of cell lines to be assessed were selected to encompass a range of productivities.

Cell Culture

The cell lines were assessed, in triplicate, in a scale-down model of Lonza Biologics' final production bioreactor process, using CDACF medium and feeds. The cell concentration and size were determined daily using a Vi-CELL automated cell counter. Product concentration was determined using Protein A HPLC.

Nutrient Analysis

Glutamate and glucose concentration in the cultures were measured daily using a Nova BioProfile 400.

mRNA Expression

Samples were taken from cultures in the exponential growth phase in the scale-down model of the final production bioreactor process. RNA was isolated. Quantitative RT-PCR was then performed using SYBR Green and a Chromo 4 PTC-200 Peltier Thermal Cycler and Opticon 4 software. Relative mRNA expression levels were calculated, normalised to β -actin.

Copy Number

DNA was isolated from samples of the cell lines. The relative heavy chain and light chain copy numbers were determined using Southern Blotting.

Statistical Analysis

To see if there was any correlation between various parameters, data were analysed using linear regression analysis. If there is no correlation between them, the analysis should return a slope of zero.

RESULTS AND DISCUSSION

Growth and Productivity

The growth and productivity of the panel was wide ranging (Tables 1 and 2 respectively).

- No correlation was found between:
 - max X_v and product concentration at harvest or specific production rate (Q_p)
 - the time integral of viable cell concentration (IVC) and product concentration at harvest or Q_p
 - doubling time (t_d) and product concentration at harvest or Q_p
- The decline in viable cell concentration after the max X_v was more pronounced for the lower producing cell lines (Figure 1)
- Conclusions that could be drawn based upon viable cell concentration and viable biomass concentration were similar (Q_p ; Table 2)

Table 1: Summary of growth data

Cell Line	Maximum Viable Cell Concentration	Harvest Viable Cell Concentration ¹	Viability at Harvest ¹	Integral of Viable Cell Concentration	Doubling Time
	10 ⁶ /mL	10 ⁶ /mL	%	10 ⁶ cell.h/mL	Hours
LB01	13.65 ± 1.27	9.72 ± 0.97	75 ± 7	2421 ± 2.18	33.2 ± 2.2
E6	6.53	5.44	90	1423	52.0
Z6	10.50 ± 0.45	5.71 ± 0.13	71 ± 4	1964 ± 59	35.9 ± 2.0
Z2	12.19 ± 1.57	6.97 ± 1.47	67 ± 19	2439 ± 100	32.7 ± 2.0
E9	9.3 ± 0.24	6.73 ± 0.70	69 ± 4	1929 ± 52	39.5 ± 3.7
Z3	10.00 ± 1.29	2.08 ± 1.25	19 ± 12	1903 ± 56	38.3 ± 2.4
Z14	11.11 ± 0.65	7.85 ± 2.17	66 ± 4	2295 ± 37	37.1 ± 7.5
Z10	10.14 ± 0.50	3.79 ± 1.21	34 ± 11	2077 ± 60	35.1 ± 0.5
Z18	10.79 ± 0.66	4.92 ± 1.25	45 ± 16	2275 ± 91	41.5 ± 0.9
Z22	12.70 ± 0.98	1.13 ± 0.08	8 ± 1	2104 ± 98	36.0 ± 2.4
Z1	11.95 ± 0.27	1.02 ± 0.76	8 ± 6	2254 ± 141	36.3 ± 1.0
CHOK1SV	9.43 ± 0.43	1.36 ± 0.22	10 ± 1	1670 ± 25	32.5 ± 2.8
NULL 8	8.97 ± 0.53	4.42 ± 0.55	50 ± 4	1612 ± 41	38.8 ± 1.3

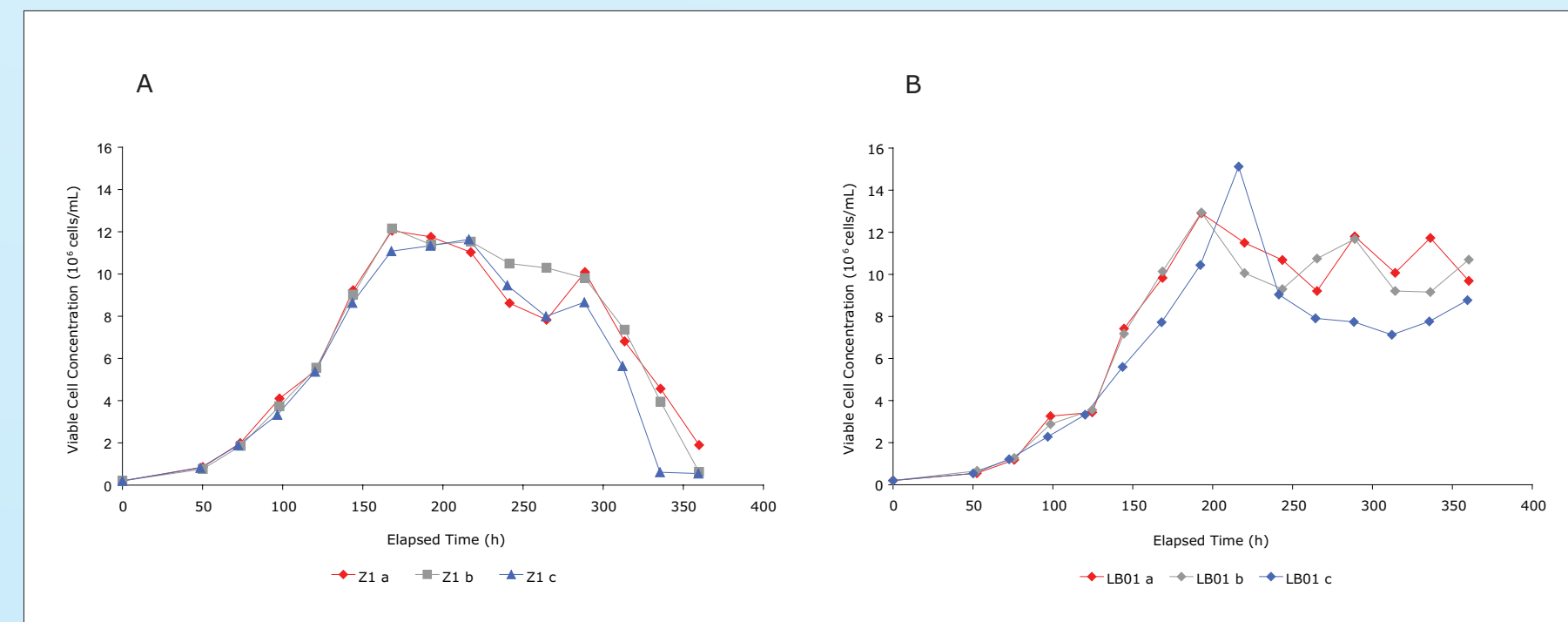
¹Harvest viable cell concentration and viability at harvest are an estimate because of lack of accuracy in Vi-CELL measurement for late overgrowth cultures

Table 2: Summary of productivity data

Cell Line	Product Concentration at Harvest mg/L	Specific Production Rate	
		Using Viable Cell Concentration pg/cell/h	Using Viable Biomass Concentration pg/10 ⁶ FL/h
LB01	1861 ± 66	0.78 ± 0.07	0.354 ± 0.052
E6	1619	1.29	0.522 ± 0.054
Z6	1303 ± 57	0.69 ± 0.06	0.282 ± 0.026
Z2	1299 ± 128	0.59 ± 0.09	0.243 ± 0.038
E9	1033 ± 72	0.60 ± 0.01	0.269 ± 0.009
Z3	991 ± 25	0.53 ± 0.03	0.243 ± 0.017
Z14	880 ± 7	0.40 ± 0.00	0.192 ± 0.007
Z10	878 ± 41	0.43 ± 0.05	0.190 ± 0.022
Z18	663 ± 16	0.30 ± 0.01	0.109 ± 0.006
Z22	534 ± 40	0.28 ± 0.02	0.137 ± 0.003
Z1	162 ± 4	0.07 ± 0.00	0.032 ± 0.002

Data were obtained from triplicate cultures, excepting E6 (single culture) and E9 (duplicate culture)

Figure 1: Example of growth profiles from triplicate cultures of (A) a low producing cell line and (B) a high producing cell line when grown in a scale-down model of the final production bioreactor process



Nutrient Analysis

Data for glutamate and glucose concentrations were used to calculate utilisation rates, taking into account supplementation from feeds (Figure 2).

These data suggest that there was often a change in metabolism around day 4: initial rates of utilisation (up to and including day 4) were different from those from day 5 onwards (post day 4). This was evident when the amounts of the components utilised were plotted against IVC (data not shown). The cause of this change is unknown. Therefore, further analysis was performed on rates calculated for (i) all data, (ii) up to and including day 4 data and (iii) post day 4 data.

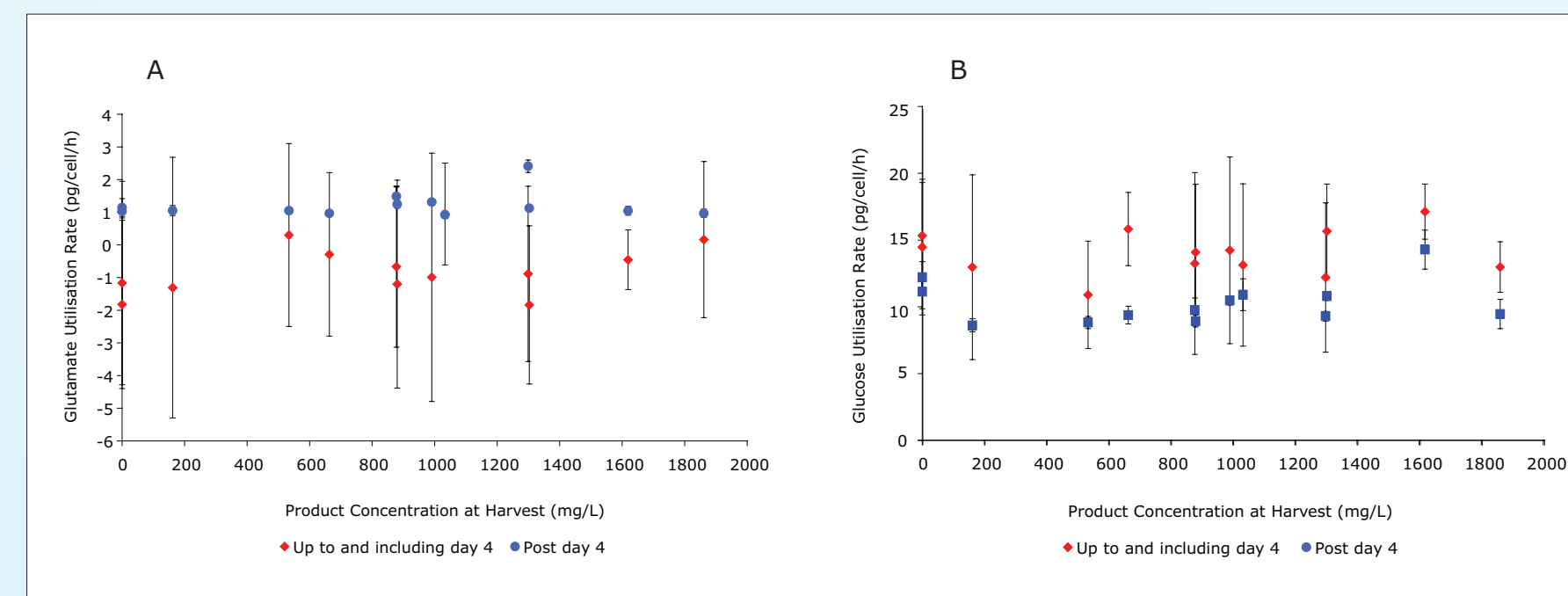
Glutamate Utilisation

- No correlation was observed between product concentration and the glutamate utilisation rate

Glucose Utilisation

- No correlation was observed between product concentration and the glucose utilisation rate

Figure 2: Analysis of (A) glutamate utilisation rate and (B) glucose utilisation rate for the panel of GS-CHO cell lines



mRNA Expression

Relative mRNA expression levels of the heavy chain and light chain were determined by quantitative RT-PCR (Figure 3).

- Correlations were observed between:
 - Heavy chain mRNA expression and product concentration (correlation coefficient = 0.66)
 - Heavy chain mRNA expression and Q_p (correlation coefficient = 0.72)
 - Light chain mRNA expression and product concentration (correlation coefficient = 0.56)
 - Light chain mRNA expression and Q_p (correlation coefficient = 0.77)

- Statistical analysis of the slopes of the regression line showed that they were significantly different from zero ($p < 0.05$)

- Higher levels of mRNA were associated with higher product concentration

This result suggests that transcription is probably a rate limiting step for productivity for this panel of GS-CHO cell lines. The literature reports work on other mammalian cell lines that appear to support (Dorai *et al*, 2006) or not support (Smales *et al*, 2004) these findings. Although analysis of the data set as a whole demonstrates that there is correlation with product concentration and Q_p , it was observed that a couple of cell lines do not appear to follow the trend. For example, cell line Z22 had similar levels of heavy and light chain mRNA expression to that of cell line E9. However, the product concentration of Z22 was almost two-fold less than that of E9. This suggests that the cause of the reduced product concentration observed for cell line Z22 occurred at some stage after transcription (i.e. translation and/or secretion).

Copy Number

The relative heavy chain and light chain copy numbers for cell lines in the panel (excluding Null 8, LB01 and Z14) were determined using Southern Blotting.

- No obvious trend exists between copy number and product concentration or Q_p (Figure 4)

Figure 3: Comparison of IgG₁ heavy chain mRNA expression and light chain mRNA expression with (A) product concentration at harvest and (B) Q_p for the panel of GS-CHO cell lines

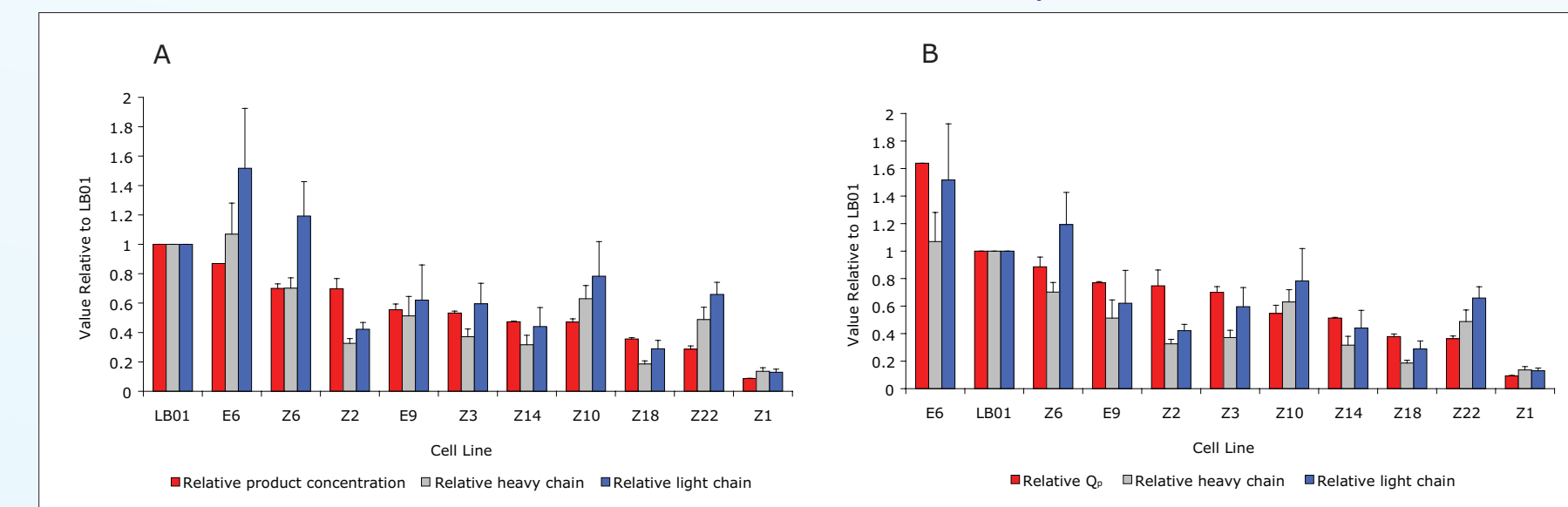
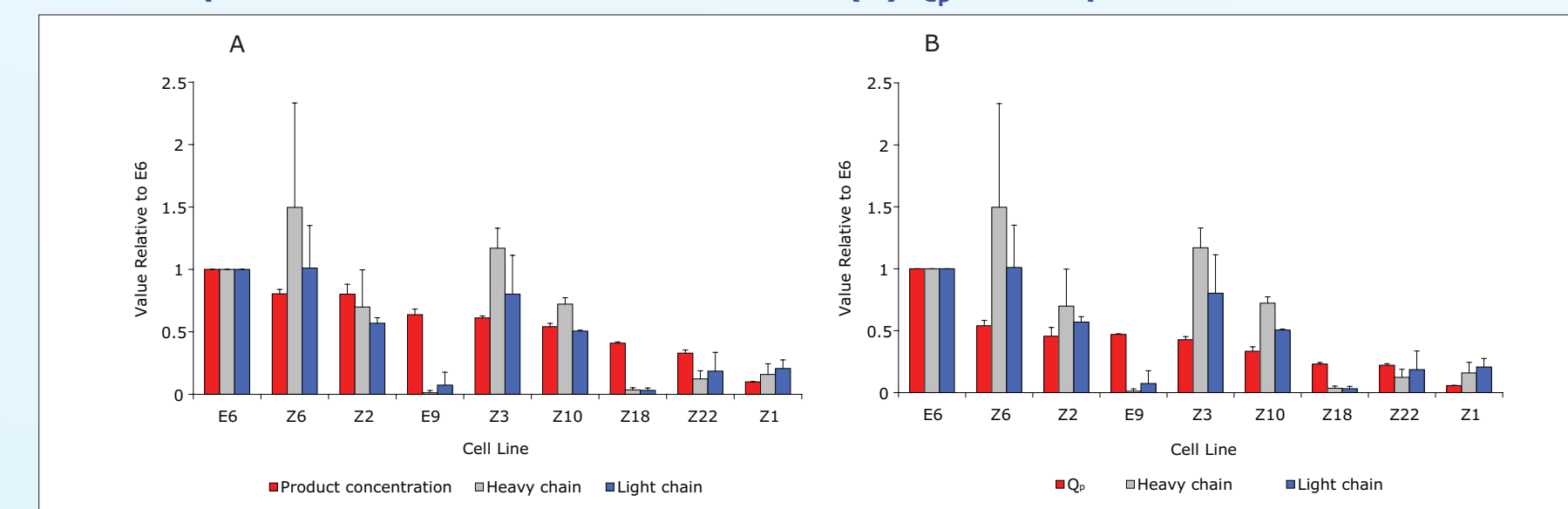


Figure 4: Comparison of IgG₁ heavy chain copy number and light chain copy number with (A) product concentration at harvest and (B) Q_p for the panel of GS-CHO cell lines



CONCLUSIONS

For this panel of GS-CHO cell lines assessed in a scale-down model of the final production bioreactor process the following observations were made:

- A correlation between productivity kinetics and mRNA expression
 - Cell lines with higher product concentrations/higher Q_p had higher levels of heavy and light chain mRNA expression
 - Suggests that transcription is probably a rate limiting step for productivity for the majority of the panel of GS-CHO cell lines
- No obvious trend between copy number and product concentration or Q_p
- No correlation between product concentration and glutamate utilisation or glucose utilisation rates
- More pronounced decline in viable cell concentration after max X_v for lower producing cell lines

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